bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Lentivirus infection of organoids for inducible knockdown and activation

ER Emma L Rawlins

Updated date: Mar 8, 2023

An abbreviated version of this protocol was published in eLIFE in Oct 2021

A functional genetic toolbox for human tissue-derived organoids

DOI: 10.7554/eLife.67886

Detailed protocol

Lentiviral transduction of organoids.

Before you start

- Pre-warm TrypLE enzyme to 37°C.
- Prepare/buy appropriate stocks of lentivirus.

Things you will need

Pipettes and filter tips (p1000, p200, p20)
1.5 ml tubes
15 ml Falcon Tubes
TrypLE Express Enzyme 1X (ThermoFisher Scientific 12605010)
30 µm cell strainers (CellTrics® filters 30um, Sysmex, 04-004-2326)
24 well plates (Greiner Bio One cat.no. 665980)
Advanced DMEM/F12 +++ (protocol 4.1) for washing.
Matrigel, Basement Membrane Matrix, Growth Factor Reduced (GFR), Phenol Red-free (BD cat.no. 356231)
Human embryonic lung self-renewal growth medium (protocol 4.2)
Human embryonic lung self-renewal growth medium (protocol 4.2) + 10 µM Rho Kinase inhibitor Y27632 (Sigma-Aldrich, Y0503)
Appropriate lentivirus stocks
Biological safety risk assessments for lentivirus use and appropriate containment level laboratory facilities.

Lentiviral transduction of organoids

Day 1

Prepare single cells

1. Culture organoids to reach 80-90% confluence.
2. Remove the medium from the organoid culture.
3. Wash the well briefly with 1x PBS.
4. Remove the PBS and add 500 µl of pre-warmed TrypLE express enzyme.
5. Triturate (pipette up and down 10 times) with P1000 pipette to break up the organoids and incubate at 37°C for 5 minutes.
6. Triturate by pipetting up and down with P200 pipette about 20 times.
7. Check that single cells have been obtained (if not, incubate a further 2-5 minutes at 37°C) and add 1 ml cold Advanced DMEM/F12 +++ to stop the reaction.
8. Filter through a 30 µm cell strainer.
9. Wash cell strainer with 2 ml of Advanced DMEM/F12 +++ and count the cell number.
10. Prepare aliquots of ~200,000 cells for each transduction. Pellet cells at 200-500 rcf for 5 minutes and resuspend in 500 µl self-renewal medium + 10 µM Rho Kinase inhibitor (Y27632).
11. Keep cells on ice until ready to transduce.

Lentivirus transduction

1. Mix lentivirus (approx. XX MOI) with organoids in 1 well of a 24 well plate.
2. Incubate at 37°C for 5-6 hours to overnight.

Seeding the transduced cells

1. Collect the transduced organoid cells into 1.5 ml tubes.

Cells may have attached to the plastic. In this case add pre-warmed TypLE and incubate at 37°C for 2 minutes to detach.

1. Fill the tubes with 1x PBS, spin 200-500 rcf for 5 minutes and discard PBS.
2. Repeat wash step 13 two more times to remove any excess lentiviral particles.
3. Resuspend the cells from each transduction in 100 µl matrigel and seed into two wells of a 24 well plate and incubate at 37°C 10-15 minutes to allow matrigel to solidify.
4. Add 600 µl human embryonic lung self-renewal growth medium + 10 µM Rho Kinase inhibitor (Y27632)..
5. After 2-3 days change the medium to human embryonic lung self-renewal growth medium.

Day 4

20. Typically start drug selection treatment, or perform flow cytometry for fluorescent protein expression, to isolate the transduced organoids.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Rawlins, E. (2023). Lentivirus infection of organoids for inducible knockdown and activation. Bio-protocol Preprint. bio-protocol.org/prep2168.
2. Sun, D., Evans, L., Perrone, F., Sokleva, V., Lim, K., Rezakhani, S., Lutolf, M., Zilbauer, M. and Rawlins, E. L.(2021). A functional genetic toolbox for human tissue-derived organoids. eLIFE. DOI: 10.7554/eLife.67886